RNA helicases in bacteria

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RNA plays a crucial role in the control of bacterial gene expression, either as carrier of information or as positive or negative regulators. Moreover, the machinery to decode the information, the ribosome, is a large ribonucleoprotein complex composed of rRNAs and many proteins. RNAs are normally single stranded but have the propensity to fold into secondary structures or anneal each other. In some instances these interactions are beneficial for the function of the RNA, but in other cases they may be deleterious. All cells have therefore developed proteins that act as chaperones or helicases to keep RNA metabolism alive.

Helicases are grouped into six superfamilies

RNA helicases form, together with DNA helicases and DNA or RNA translocases, 6 superfamilies (SF) that can readily be distinguished based on their amino acid sequence [1]. Within their conserved core they harbour one or two domains that resemble the recombination protein RecA and are therefore referred to as RecA-like domains. SF1 and SF2 are composed of mono-or oligomeric enzymes and contain DNA helicases and all RNA helicases known so far. Superfamilies 3 to 6 are composed of multimeric, ringforming structures (for review see [1]). With the exception of SF5 that is composed of a single protein, the well characterized termination factor Rho, all other superfamilies 3-6 contain DNA helicases, translocases and AAA+ proteins (ATPases Associated with various Activities). In the following, we will describe what is known about RNA helicases in bacteria.

Superfamily 1 RNA helicases

The superfamily 1 is composed of three families of helicases, the UvrD/PcrA/Rep family, the Pif1/RecD family, and the Upf1 family. The UvrD/PcrA/Rep family and the Pif1/RecD family contain DNA helicases that are involved in DNA metabolism in prokaryotes and eukaryotes. The Upf1 family has so far only been described for eukaryotic cells. Upf1 proteins were found in various screens to be implicated in processes that involve DNA or RNA.

Upf1 family

The Upf1 protein (upf = up frameshift) was originally identified in yeast as a suppressor of frameshift mutations and found to be involved in nonsense mediated RNA decay (NMD) [2]. Upf1 from yeast and human were shown to be efficient 5'-3' ATP dependent RNA helicases and involved in disassembly of RNP complexes *in vivo*, prior to degradation of the RNA [3]. However Upf1 could also use DNA as a substrate and aside its function in NMD, it was also found to participate in the nucleus in DNA replication as well as telomere maintenance [4]. Thus, it seems that Upf1 has a split personality and possesses different functions.

Analysis of a number of bacteria from different phyla showed that a few encode proteins with various degrees of homology with the core region of yeast Upf1 (amino acids 361 -840) (Figure 1), but to the best of our knowledge, none of these genes has been described in the literature and it remains to be determined whether they function as RNA helicases and/or rather as DNA helicases.

Superfamily 2 RNA helicases

Superfamily 2 contains several families of RNA helicases (DEAD, DEAH/RhA, Ski2-like, viral DExH), but harbors also DNA helicases (Rad3/XPD, RecQ, RecG) and translocases (type I restriction enzymes; SWI/Snf-like proteins) [5]. The conserved motifs of the superfamily 2 proteins are similar throughout, but sufficiently different to clearly attribute a given protein to one or another family (Figure 1).

DEAD-box proteins

DEAD-box RNA helicases represent the largest family of RNA helicases. Although referred to as helicases, they are characterized by a poor helicase activity able to unwind only short RNA or DNA-RNA duplexes and perform local strand separation in a non-processive manner. Elucidation of their mechanism of action was obtained mainly by the structural analysis of the *Drosophila* DEAD-box RNA helicase Vasa [6]. When bound to a double stranded substrate, the protein induces a kink in the nucleic acid inducing a local strand separation, that can be further used for either protein-binding to one of the strands, or annealing with another nucleic acid, for example a small regulatory RNA. Moreover, analysis of the ATPase activity has shown that only ATP binding is required for unwinding, but ATP hydrolysis is necessary for recycling of the protein [7] and a loading strand is required in some instances, but does not serve as translocation track for the helicase [8]. Such an activity confers a broad variety of activities to DEAD-box RNA helicases, which in some cases are not related to RNA unwinding. Indeed, in addition to these strand separation events, DEAD-box proteins were also shown *in vitro* to displace proteins from RNA [9], to form stable complexes with RNA [10] or to facilitate annealing of two complementary RNAs [11]. Many of the bacterial DEAD-box proteins were found serendipitously in a variety of screens and selections (Table 1) or identified by a targeted search for conserved motifs. Nevertheless in many cases the function of the DEAD-box protein is not clear. Only few bacterial DEAD-box proteins have been studied and their activity is so far restricted to ribosome biogenesis, RNA decay, and translation initiation. In a phylogenetic analysis of the DEAD-box proteins, we found that most bacteria, with few exceptions, encode at least one DEAD-box RNA helicase and some up to 12 different members [12]. The bacterial DEAD-box proteins used in this study clustered into 25 different groups but few of these groups, mainly from *E. coli*, have been studied so far, which could indicate that new functions or properties of a so far unstudied group may be discovered. In the following section we will describe the processes in which some information on the function of RNA helicases is available.

mRNA turnover

One of the best characterized bacterial DEAD-box proteins is RhlB from *E. coli*. It facilitates the degradation by the exoribonuclease PNPase of RNA intermediates harboring strong secondary structures [13]. The coordination of their activity requires their association with the essential endoribonuclease RNase E that functions as the scaffold of the RNA degradosome and stimulates the activity of RhlB, although a RNase E independent RhlB-PNPase complex was reported in the literature [14,15]. RNA degrading complexes associating ribonucleases with helicases appear to be a ubiquitous concept exemplified in eukaryotes by the exosome that also contains an RNA helicase, albeit a Ski2-like protein [16]. In *Bacillus subtilis* and *Staphylococcus aureus* the degradosome is composed of a different set of ribonucleases and the DEAD-box protein CshA [17,18]. First described in *S. aureus* as involved in biofilm formation [19], CshA is now known to play a general function in mRNA decay [20]. The decreased biofilm production is the consequence of an inefficient degradation of the *agr* mRNA in a $\Delta cshA$ strain (Figure 2a) [21]. However, the structure(s) of the degradosome or its subcomplexes involved in RNA turnover are not known and more importantly neither the molecular signatures of the target RNAs nor the partner(s) that require or regulate the activity of CshA are known.

Ribosome biogenesis

Hints for a role of DEAD-box proteins in ribosome biogenesis were given since their first description in *E. coli*. Two of the five DEAD-box proteins in *E. coli*, SrmB and CsdA (originally named DeaD and subsequently renamed CsdA [22,23], we use the nomenclature CsdA, to avoid confusion with the wrongly annotated DeaD from *B. subtilis*, that in fact should be YxiN [24]), were originally isolated as multicopy suppressors of temperature sensitive mutations in ribosomal protein genes L24 and S2, respectively [22,25]. Since then, a careful analysis has shown that both SrmB and CsdA are required for 50S biogenesis, especially at low temperature in *E. coli* [26,27]. Moreover, an elegant genetic screen from the Dreyfus laboratory identified the sites of action of SrmB to be present in the rRNA, providing new insights onto the molecular function of SrmB (Figure 2b) [28]. Intriguingly, the authors reported that SrmB would not need its ATPase function for this activity. Two other DEAD-box proteins of *E. coli*, RhlE

and DpbA also appear to act in ribosome biogenesis. The latter one represents a unique case as DpbA is specifically activated by a specific part of the 23S RNA [29]. Intriguingly, the deletion of *dpbA* does not affect ribosome biogenesis, suggesting that its function is either not essential or could be bypassed under the tested conditions. Nevertheless, overexpression of a DbpA with a mutation in motif VI, involved in ATP hydrolysis, leads to growth and ribosome biogenesis defects [30]. Similarly, *rhIE* deletion does not lead to a growth deficiency, but genetic interactions with SrmB and CsdA suggested a role in ribosome biogenesis [31]. It is interesting to note that bacteria that have a large number of DEAD-box proteins have several copies that are most closely related to RhIE [12]. Whether these would function in ribosome biogenesis or in other processes is unknown. Studies in *B. subtilis* or *Listeria* also suggested that DEAD-proteins function in ribosome biogenesis although a much more in-depth analysis will be required to confirm their function [32,33].

Translation initiation

RNA helicases are known to play important roles for translation initiation in eukaryotes to allow binding and scanning of the small ribosomal subunit. In bacteria, the binding to the ribosome binding sequence (RBS) has been shown to be an important step that can easily be regulated by secondary structures or antisense RNA. Surprisingly few examples were reported in which DEAD-box proteins alter the accessibility of the SD sequence [34-36]. CsdA was shown to be required for the annealing of a small regulatory RNA, DsrA, to the 5' UTR of *rpoS* to free the RBS sequence [34]. It also has been shown to stimulate the translation of the transcriptional regulator UvrY, which is regulated by an extended putative secondary structure. In this case the CsdA requirement is abolished if mutations are introduced into a stem structure, but re-established if complementary mutations restore the stem-loop structures [36]. In addition to UvrY the authors also identified 38 other RNAs that were enriched in a high throughput sequencing of RNAs cross-linked to a tagged CsdA suggesting a more wide function for CsdA in translational control. A very recent publication shows that CsdA from *Pseudomonas* is required for the modification of a secondary structure to efficiently express ExsA, a transcription factor of the TTSS (type three

secretion system). Both, deletion of the 5'UTR or replacement of the beginning of the protein coding sequence of ExsA from heterologous systems, abolish the formation of the secondary structure and the requirement of CsdA (Figure 2c) [35]. In these cases it is not known whether the helicase acts alone and how it is directed to its target. Could this be through an association with the small ribosomal subunit?

DEAH-box proteins

DEAH-box proteins are highly similar to DEAD-box proteins but show some distinct motifs, such as the DEAH motif, the signature of this family. They have been studied extensively in baker's yeast where they were identified as factors in splicing and ribosome biogenesis. They were reported to perform 3'-5' unwinding, but can also be involved in protein dissociation from a substrate RNA (e.g., [37]). They are thought to perform biochemical activities that are similar to those of DEAD-box proteins, but DEAH family proteins are promiscuous for the NTP hydrolysis. Like in the case of DEAD-box proteins, DEAH-box proteins are stimulated by accessory proteins. In the case of Prp43, the interaction and stimulation by the G-patch proteins Ntr1 and Pfa1 for ribosome biogenesis and pre-mRNA splicing, respectively, is a beautiful example how accessory proteins can target and regulate RNA helicases in different cellular processes [37,38]. Relatively little is known about DEAH-box proteins in bacteria. A phylogenetic tree of bacterial DEAH proteins showed three different groups called HrpA-like, HrpB-like and Z5898-like found exclusively in proteobacteria and actinobacteria [12]. Only HrpA-like and Z5898-like proteins have been studied experimentally. HrpA has been shown to be required for the processing of the *daa* operon encoding a fimbrial adhesin. The structural gene of fimbria encoded by *daaE*, which is the last gene of the operon, is needed in greater abundance than the preceding genes. To differentially express these genes, the full length mRNA is cleaved in the penultimate gene, in a translation dependent manner, by a so far unidentified endonuclease. The upstream part of the mRNA is then rapidly degraded, whereas the downstream part, encoding *daaE*, is stabilized by a 3' hairpin structure. Using a clever GFP sorting system, mutants deficient in the cleavage were enriched and one mutation was found to locate in

HrpA (Figure 2d) [39]. In another report, deletion of HrpA or mutations affecting the ATPase or helicase activity in *Borrelia burgdorferi* resulted in loss of infectivity in mice [40]. This study proposed that HrpA has global function in gene regulation as indicated by a partial proteome analysis revealing 90 down- and 97 up-regulated proteins. The third bacterial DEAH-box protein described in the literature is encoded by a pathogenicity island (called motility associated O - MAO - island) in *E. coli* O157:H7, but also present in two unrelated *E. coli* and two *Salmonella* strains [41]. Mutation of the Z5898 DEAH-box helicase gene resulted in down regulation of flagellin and reduced motility. Intriguingly, the authors show that *fliC* expression is down regulated and a GFP fusion indicated that this occurs at the transcriptional level, but further analysis is required to fully understand the role of Z5898.

Ski2-like proteins

The Ski2-like family is named after a yeast protein originally identified as conferring a super killer phenotype if mutated in a strain containing linear dsRNA virus like particles. In baker's yeast, the family is composed of Ski2 itself, its homologue Mtr4, Brr2, and Slh1 [42]. The structural analysis was very much facilitated by using the archaeal Hel308 DNA helicase [43]. Ski2 and Mtr4 are helicases of the cytoplasmic and nuclear exosome complexes, respectively. Brr2 is involved in pre-mRNA splicing and Slh1 is involved in transcription and translation. Ski2-like proteins are also composed of two RecA domains, with a wedge in the second domain to separate the strands when the helicase translocates with 3'-5' polarity using a putative inchworm mechanism, helped by a ratched domain [44]. An analysis of bacterial genomes reveals that closest homologs of Ski2-like proteins are found mainly in Cyanobacteria and Actinobacteria. Hely from *Mycobacterium smegmatis* was recently shown to be an RNA-stimulated ATPase and an RNA:RNA or RNA:DNA helicase [45]. Nevertheless, to the best of our knowledge, no bacterial Ski2-like protein has been characterized *in vivo* so far.

Non-DEAD and non-DEAH RNA unwinding proteins

In addition to the RNA helicases characterized by the conserved motifs including

the Walker A (I) and Walker B (II) motifs, there are also a variety of other proteins that modify RNA structures that do not belong to these families. Here we describe two examples, RNase R or CsrA.

RNase R was originally described as *vacB* virulence factor from *Shigella flexneri* [46]. It is 60 % homologous to RNase II and deletion of RNase R is synthetically lethal with a PNPase deletion [46], which both have a 3'-5' exonuclease activity. Interestingly RNase R popped up in a suppressor screen for a *csdA* helicase mutant [47]. In contrast to PNPase and other exoribonucleases, RNase R can efficiently degrade structured RNA and has, in addition to its nucleolytic activity, an RNA helicase activity. As a complete surprise, the Deutscher laboratory reported recently the presence of an ATPase motif II at the N-terminus and a motif I at the C-terminus and showed that binding to ATP was required for its helicase activity [48]. The mutational analysis of these motifs showed that they are indeed necessary for ATPase and helicase and affect ribonuclease activity, especially at low temperature.

Another example of an RNA structure modifying protein is CsrA, involved in adaptation to many different environmental changes (for a recent review, see [49]). CsrA is a small peptide that generally binds to a sequence containing a GGA motif, explaining its preference for ribosome binding sequences, and thereby often functioning as a translational repressor. CsrA also binds to the small RNAs CsrB and CsrC, that play an important role in CsrA mediated regulation by titrating this RNA binding protein. Very recently, it has been shown that CsrA can destabilise an RNA secondary structure that normally hides the termination factor Rho binding site *rut* and thereby allows transcription termination of the *pgaABCD* operon, involved in extracellular polysaccharide adhesion synthesis [50]. Interestingly, CsdA and SrmB regulate CsrB and CsrC expression by stimulating translation initiation of UvrY (see above), the transcriptional activator of CsrB and CsrC [36].

Conclusion

Bacterial DEAD-box proteins were discovered in many different screens and in some cases their function in a particular process could be identified. Although their function in these processes fit perfectly with their predicted biochemical

activity, such as unwinding secondary structures for RNA degradation or rearranging RNP complexes in ribosome biogenesis, in most cases the molecular function has not been defined precisely. With the availability and combination of state of the art techniques such as crosslinking methods, RNA deep sequencing, and sophisticated genetic screens it will be hopefully possible to delineate their molecular functions more precisely.

In general and even if DEAD-box protein are not likely to be redundant, their inactivation does not lead to a severe phenotype under standard laboratory conditions, they are crucial in some growth or stress conditions suggesting a role in more competitive environments. Moreover, some DEAD-box proteins, such as exemplified by CsdA, are likely to be involved in different processes, possibly by interacting with different mediators.

Another remaining and intriguing question is the absence of DEAD-box proteins in some bacteria. This could be explained for bacteria living at elevated temperature or for Chlamydia by their intracellular growth at moderate temperature. But how do *Borrelia* manage to grow without these proteins at lower temperature? Have they evolved the DEAD-box protein targets in such a manner that these proteins are no longer required, or do they use other proteins? In contrary, do bacteria that have multiple DEAD-box protein genes (up to 12 for *Shewanella*) use different DEAD-box proteins for different growth conditions? And finally, it is striking that many DEAD-box proteins were identified in genetic screens, but so far Upf1-like and Ski2-like proteins have only been identified by bioinformatics. Future work will be needed to elucidate the molecular functions played by these proteins from different families..

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Figure legends

Figure 1: Conserved motifs within the different helicase proteins allow them to cluster within families.

Proteins from yeast and humans (top line for each family) are very similar to those from bacteria (bottom line for each family). Red residues were fully conserved amongst the proteins analysed in a given family. For the Upf1 family 13 eukaryotic and 100 bacterial sequences were aligned and residues were given if 8 and 80 or more sequences were identical, respectively. For DEAD box proteins, residues were indicated if 35 or more out of 61 eukaryotic sequences and 46 out of 77 bacterial sequences were identical. For DEAH-box proteins residues are indicated if 16 out of 23 and 60 out of 100 or more sequences were identical. For Ski2-like proteins, residues were retained when 6 out of 10 and 80 out of 99 or more sequences were identical, for eukaryotic and bacterial proteins, respectively. For bacterial Ski2-like proteins the similarity was very high, since most of the proteins were from Cyanobacteria or Actinobacteria, and only two sequences from Chloroflexi, one sequence from Fibrobacteres, one sequence from Chlamydiae, and one sequence from Proteobacteria.

Figure 2a: The DEAD-box RNA helicase CshA is involved in RNA degradation.

A genetic screen for mutants with decreased biofilm formation identified CshA from *S. aureus*. In absence of CshA, the *agr* mRNA is stabilized. This leads to increased RNA III levels through activation of the quorum sensing system, composed of AgrB, the transporter, AgrD, the signaling peptide or auto-inducer, AgrC, the sensor, and AgrA, the transcriptional activator of the *agr* and the RNA III promoters. This regulatory RNA is known to regulate several target genes positively or negatively leading to reduced expression of surface proteins, and increased expression of secreted proteins such as hemolysins.

Figure 2b: SrmB prevents deleterious annealing of 23S and 5S rRNAs.

This genetic screen used an *E. coli* strain in which all 7 rRNA operons were deleted and the only copy was present on a plasmid. This strain also contained a deletion of the *srmB* gene, rendering the strain cold sensitive. Screening for mutations that allowed growth at a low temperature identified mutations in the 23S rRNA or the 5S rRNA genes. Interestingly, the mutations were located in repeated sequences that are complementary between the 23S and the 5S rRNA.

Figure 2c: CsdA/DeaD was identified in a screen for mutations that reduce cytotoxicity of *Pseudomonas*.

In absence of CsdA the ExsA gene expression is reduced, resulting in delayed cytotoxicity. If the 5' upstream region or the beginning of the reading frame is exchanged with the sequence from other but related genes the expression becomes CsdA independent.

Figure 2d: The production of sufficient quantities of fimbrial proteins requires a processing event of the *daa* mRNA.

Under normal conditions, the *daa* operon is cleaved in a translation dependent

manner within the *daaP* gene. A fusion of a GFP reporter gene in its 3' region with the *daaP* containing cleavage sequence and the 3' protecting secondary structure will show reduced GFP activity if the transcript is processed [39]. If the processing cannot take place, as for example in absence of HrpA, the 3' situated secondary structure will protect the entire mRNA and increase the GFP expression.

Table 1: In vivo phenotypes associated with mutants in RNA helicases

Protein	In vivo phenotypes	Organism	Reference
CrhC	Induced if shifted to 20°C, induction is light dependent	Anabaena sp PCC 7120	[51]
	Membrane associated	Anabaena sp PCC 7120	[52]
CrhR	Induced by light, not detectable in the dark	Synechocystis sp. PCC 6803	[53]
	Altered morphology and surface	Synechocystis sp. PCC 6803	[54]
	Autoregulation in response to temperature stress	Synechocystis sp. PCC 6803	[55]
CsdA	Multicopy suppressor of <i>rpsB^{ts}</i> (S2 protein)	<i>E. coli</i>	[22]
	Ribosome biogenesis, 50S	<i>E. coli</i>	[26]
	Cold shock induced	<i>E. coli</i>	[23]
	Cold sensitive	<i>E. coli</i> , <i>L. pneumophila</i> , <i>Y. pseudotuberculosis</i>	[26,56,57]
	Synthetic interaction with <i>rne^{ts}</i>	<i>E. coli</i>	[58]
	<i>csdA::Tn10</i> suppresses lethality of Δrne	<i>E. coli</i>	[59]
	Assembles with cold induced degradosome	<i>E. coli</i>	[58]
	Stabilisation of <i>cspA</i> mRNA at 15°C in a <i>csdA</i> mutant	<i>E. coli</i>	[47]
	Synthetic enhancement with $\Delta RNase R$	<i>E. coli</i>	[47]
	Cold sensitivity can be partially suppressed by overexpression CspA or RNase R	<i>E. coli</i>	[60]
	Overexpression of CsdA permits efficient translation of CAT reporter	<i>E. coli</i>	[61]
	Required for TTSS expression	<i>Pseudomonas</i>	[35]
	Required for RpoS expression	<i>E. coli</i>	[34]
	Stimulates UvrY translation	<i>E. coli</i>	[36]
	Stimulates SirA translation	<i>Salmonella</i>	[36]
CshA	Identified in a screen for exported proteins	<i>S. pneumoniae</i>	[62]
	Required for aggregation of bacteria	<i>L. reuteri</i>	[63]
	Increased resistance to oxidative stress	<i>C. perfringens</i>	[64]
	Required for catalase activity	<i>E. faecalis</i>	[65]
	Cold shock response	<i>B. subtilis</i>	[66,67]
	Sediments with polysomes	<i>B. subtilis</i>	[68]
	Defect in ribosome biogenesis	<i>L. monocytogenes</i>	[32,33]
	Stimulates biofilm formation	<i>S. aureus</i>	[19]
	Interaction with degradosome	<i>B. subtilis</i> , <i>S. aureus</i>	[17,18,20]
	Cold sensitive	<i>B. subtilis</i> , <i>B. cereus</i> , <i>S. aureus</i> , <i>L. monocytogenes</i>	[21,69,70] [71]
	Reduced motility	<i>L. monocytogenes</i>	[71]
CshB	Reduced growth rate	<i>U. parvum</i>	[72]
	Cold sensitive	<i>B. cereus</i> , <i>B. subtilis</i> , <i>S. aureus</i>	[32,73,74]
	Upregulation of phenolic acid decarboxylase (<i>padA</i>)	<i>L. plantarum</i>	[75]
	Reduced expression in late exponential and stationary phase	<i>B. subtilis</i>	[17]
DbpA	R331A is dominant negative when overexpressed in a wild-type background	<i>E. coli</i>	[76]
RhlB	Accumulation of degradation intermediates <i>in vivo</i>	<i>E. coli</i>	[13]
	Required for axenic growth	<i>B. bacteriovorus</i>	[77]
RhlE	Multicopy suppressor of $\Delta csdA$	<i>E. coli</i>	[31,47]
	Its overexpression exacerbate the cold sensitivity of $\Delta srmB$	<i>E. coli</i>	[31]
	Decreased survival after freezing	<i>C. crescentus</i>	[78]
	Upregulated in the cold	<i>P. arcticus</i> 273-4	[79]
	Reduced pathogenicity in Nematodes	<i>P. luminescens</i> LN2	[80]
SrmB	Multicopy suppressor of L24	<i>E. coli</i>	[25]
	Cold sensitive	<i>E. coli</i>	[27]
	Defect in ribosome biogenesis	<i>E. coli</i>	[27]

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Figure 1

		Q or 0	I	Ia	Ib	Ic	II	III	IIIa	IV	IVa	V	Va	Vb	VI
Upf1-like		LN•Q••A	GPPGTGKT	L•C•PSN	RLG	T	DEA•Q	GDH•QL	L••QYRM	IG•ITPY••Q		D•FQG•E	I••S•VR•N	•GFL	RR•NVA•TRA
		LN•Q••A	GPPGTGKT	LVCA•SN	RIG	T	DEAAQ	GDH•QL	L••QYRM	•G•ISPY••Q		D•FQGQE	I••S•VR•N	IGFL	RR•NVA•TRA
DEAD	F	G•••PTPIQ	A•TGSGKT	PTRELA•Q	GG	TPGRL•D	DEAD••L	FSAT		IVF	HG•••Q••R	L••TD	AARG•D	V•N•D	Y•HR•GRTGR
	F	GF•••TPIQ	AQTGTGKT	PTRELA•Q	GG	TPGRL•D	DEAD•M•	FSAT		IVF	•G•••Q•KR	LVATDV	AARGLD	V•N•D	YVHRIGRTGR
DEAH		LP	G•TGSGKT	TQPRR•AA	V••E	T•G•L•R	DE•HER	SAT		G••L•FL•G	L••••Q•••F	TNIAETS•T	V•D•G••K	S•A	QR•GRAGR
		LP	GETGSGKT	TQPRR•AA	I••E	TDGILLA	DEAHER	SAT		GD•L•F••G	LS••EQ•R•F	TNVAETSLT	VID•GTAR	SQA	QRKGRCGR
Ski2-like		Q	A•T••GKT	P•KAL	TGD	MTTE	DEVH	LSAT	RP•PL	IVF	HH•G	E•LF••G	G•NMP		QM•GRAGR
		D•FQ	APTG•GKT	P•KAL	TGD	MTTE	DEVH	LSAT	RPVPL	FIF	HHAG	E•LF••G	G•NMP		QL•GRAGR

Figure 2a

Figure 2b

Figure 2c

Figure 2d

RNA helicases